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## Review

## Growing on calcareous soils and facing climate change

Thibaut Bontpart <sup>1</sup>, Alizée Weiss <sup>2</sup>, Denis Vile <sup>3</sup>, Frédéric Gérard <sup>4</sup>, Benoît Lacombe <sup>1</sup>, Jean-Philippe Reichheld <sup>5</sup> and Stéphane Mari <sup>1,\*</sup>

**Soil calcium carbonate (CaCO<sub>3</sub>) impacts plant mineral nutrition far beyond Fe metabolism, imposing constraints for crop growth and quality in calcareous agrosystems. Our knowledge on plant strategies to tolerate CaCO<sub>3</sub> effects mainly refers to Fe acquisition. This review provides an update on plant cellular and molecular mechanisms recently described to counteract the negative effects of CaCO<sub>3</sub> in soils, as well as recent efforts to identify genetic bases involved in CaCO<sub>3</sub> tolerance from natural populations, that could be exploited to breed CaCO<sub>3</sub>-tolerant crops. Finally, we review the impact of environmental factors (soil water content, air CO<sub>2</sub>, and temperature) affecting soil CaCO<sub>3</sub> equilibrium and plant tolerance to calcareous soils, and we propose strategies for improvement in the context of climate change.**

### CaCO<sub>3</sub> disturbs plant mineral nutrition and homeostasis in calcareous soils

**Calcareous soils** (see [Glossary](#)), which cover approximately 30% of the world's land area, including cultivated areas [1,2], contain **calcium carbonate** (CaCO<sub>3</sub>) inherited from parent material (primary carbonates) and/or precipitated in place (secondary carbonates). CaCO<sub>3</sub> can also form after the release of Ca oxalate-containing plant tissues (mostly through litterfall) and their oxidation and degradation by oxalotrophic bacteria and fungi [3,4]. According to the Food and Agriculture Organization, 'calcareous soils have often more than 15% CaCO<sub>3</sub> in the soil.' This percentage corresponds to the threshold above which a soil horizon is qualified as calcic (i.e., accumulating secondary carbonates) and not continuously cemented [5]. CaCO<sub>3</sub> is subject to fast dissolution-precipitation reactions and thereby actively participates in the carbon (C) biogeochemical cycle at the human scale [1]. CaCO<sub>3</sub> dissolution-precipitation depends on a range of biotic (e.g., microbiota respiration) and abiotic (e.g., soil composition and moisture) factors [6]. Calcareous soil pH is mainly buffered by reactions with CaCO<sub>3</sub> and air CO<sub>2</sub> and is close to 8 [7]. Above pH<sub>water</sub> ≥ 8.7, it is considered that CaCO<sub>3</sub> is not the main buffer and that the horizon is ultra-basic (noncalcic) due to the presence of other carbonate salts such as Na<sub>2</sub>CO<sub>3</sub> and MgCO<sub>3</sub> [5]. Hence, calcareous soils can be distinguished from other alkaline soils such as saline and dolomitic soils where other exchangeable cations (Na and Mg, respectively) are more abundant. CaCO<sub>3</sub> dissolution releases calcium (Ca<sup>2+</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>) ions ([Figure 1](#), Key figure). Ca<sup>2+</sup> is adsorbed on soil constituents, especially clay minerals and organic matter, where it can compete with adsorbed protons. Dissolved carbonates interact with protons to form another **dissolved inorganic carbon** (DIC) species, the bicarbonate ion (HCO<sub>3</sub><sup>-</sup>), which is the dominant aqueous species at pH 8. This proton consumption is responsible for the pH increase of the soil solution. As a result, calcareous soil conditions impose multiple constraints for plant nutrition, especially for maladapted crops in agricultural systems. First, plants have to deal with elevated Ca and carbonate concentrations. Second, alkaline conditions affect **ion speciation** and consequently macro- and micronutrient availability for plants (i.e., **nutrient phytoavailability**). Indeed, alkaline conditions restrict the availability of plant nutrients such as phosphorus (P), iron (Fe), manganese (Mn), zinc (Zn), and,

### Highlights

Soil calcium carbonate (CaCO<sub>3</sub>) affects plant nutrient acquisition and consequently shoot mineral homeostasis leading to chlorosis and yield losses in maladapted crops.

Different tolerance strategies have been identified, notably Ca flux regulation, biomineralization, and secretion of root exudates to remobilize unavailable soil nutrients.

Recent intraspecific genomic analyses revealed that wild species detain a pool of alleles, notably for ion transporters, that could be exploited to improve plant tolerance to calcareous soil.

Contrary to soil water content, the effects of air and soil temperature and CO<sub>2</sub> concentration on plant performance in calcareous soil are largely unknown.

Metabolic engineering in *Arabidopsis thaliana* to increase Ca biomineralization is an attractive strategy to improve plant tolerance to CaCO<sub>3</sub> in the context of climate change.

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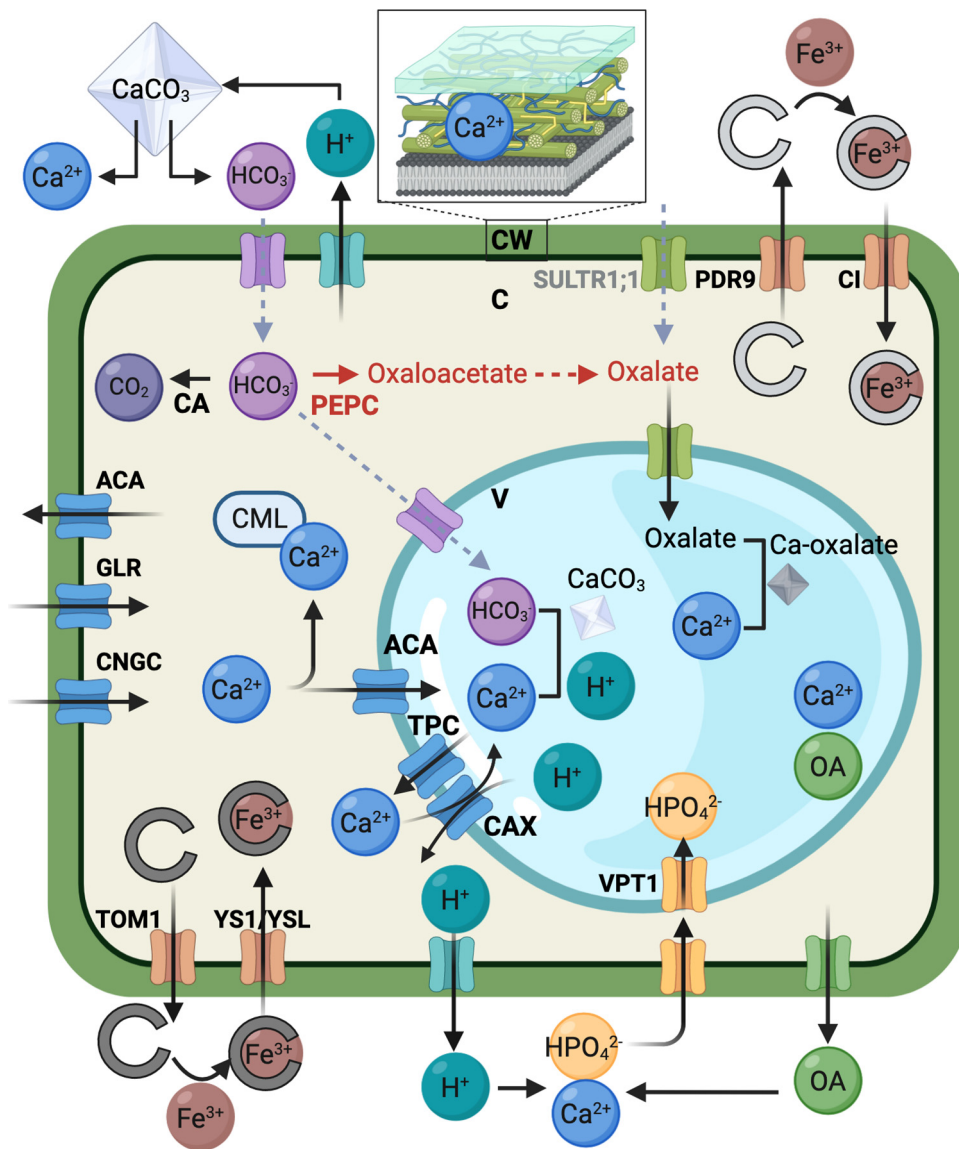
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**Key figure**

Candidate molecular actors involved in plant tolerance to calcareous soil



**Glossary**

- Calcareous soils:** soils containing more than 15% CaCO<sub>3</sub> (according to the Food and Agriculture Organization).
- Calcicole plant:** plant that preferentially grows on calcareous soils.
- Calcification:** formation of CaCO<sub>3</sub>.
- Calcifuge plant:** plant that avoids calcareous soils.
- Calcium carbonate:** calcium carbonate (CaCO<sub>3</sub>) is a mineral made of carbonate (CO<sub>3</sub><sup>2-</sup>) and calcium (Ca<sup>2+</sup>).
- CCal:** carbon calcium inclusions are cellular solid deposits made of calcium and carbonaceous ion (mainly oxalate and carbonate) formed in plants.
- Chlorosis:** leaf yellowing due to the lack of chlorophyll, a green pigment involved in plant photosynthesis.
- Cluster roots:** cluster or proteoid roots are plant lateral roots that developed clusters of shortly spaced, highly dense rootlets, as an adaptation to nutritional shortage.
- Dissolved inorganic carbon:** dissolved inorganic carbon (DIC) is the sum of inorganic carbon species: the carbon dioxide (CO<sub>2</sub>), the bicarbonate (HCO<sub>3</sub><sup>-</sup>), and the carbonate (CO<sub>3</sub><sup>2-</sup>) ions found in solution.
- Genome-wide association studies:** genomics approach focusing on association between SNP and phenotype.
- Genomic variation:** differences between genomes can involve SNP, nucleotide insertion or deletion (indel), or a larger portion of the genome such as copy number variant (CNV), resulting in duplication events.
- Idioblast:** cell specialized in the storage of specific compounds such as CaCO<sub>3</sub>.
- Ion speciation:** chemical identification of the molecular complexes formed between ions and ligands. The speciation is influenced mostly by ligand concentration, redox potential, and pH.
- Lignification:** formation of complex organic polymers (lignin) in the cell apoplast.
- Nutrient phytoavailability:** availability of soil nutrients for absorption by plant roots.
- Root exudates:** substances secreted by roots (e.g., protons, organic acids, phenolic compounds).
- Sodium bicarbonate:** sodium bicarbonate (NaHCO<sub>3</sub>) is a carbonate salt, notably abundant in saline calcareous soils.

**Figure 1.** CaCO<sub>3</sub> buffering effect favors proton consumption and Ca and bicarbonate enrichment in soil. Bicarbonate transporter has not been identified in plants. Its conversion into CO<sub>2</sub> and oxaloacetate is catalyzed by CA and PEPC, respectively. The production of oxalate from oxaloacetate can be catalyzed using enzymes encoded by bacterial genes from the oxalate biosynthetic operon [57]. Oxalate could be imported in the cytosol via SULTR1;1 transporter [69]. Once imported in the vacuole, oxalate can interact with Ca to form Ca-oxalate crystals. CNGC and GLR import Ca into the cytosol, whereas ACA ensures both Ca vacuolar storage and excretion. TPC takes part in Ca flux from the vacuole to the cytosol, where it can bind CML. Ca interaction with HCO<sub>3</sub><sup>-</sup> in the vacuole leads to CaCO<sub>3</sub> formation and releases proton. At the vacuole membrane, CAX simultaneously ensures Ca entry and proton exit. Ca is also stored in complexes formed with OA in the vacuole or pectin in CW. OA and proton excretion favors the release of phosphate (P) from insoluble Ca-P minerals. VPT1 ensures P storage in the

(Figure legend continued at the bottom of the next page.)

to a lesser extent, potassium (K) and magnesium (Mg) [8]. Third, Ca interaction with other nutrients can lead to the formation of unavailable precipitates [9]. The low availability and resulting deficiency in nutrients lead to changes in morphology, physiology, and yield, these responses being dependent on  $\text{CaCO}_3$  content, plant species, and varieties [10]. Maladapted plants are described by reduced shoot biomass and in some cases display leaf **chlorosis**. However, the lower nutrient availability induced by  $\text{CaCO}_3$  in the soil solution does not systematically result in lower shoot nutrient concentrations, even in chlorotic leaves [11], reflecting adaptive strategies to mobilize, absorb, and allocate soil nutrients. Nevertheless, similar nutrient concentrations at the whole-plant level can mask changes in their compartmentalization and use in shoots, notably for enzymes [12,13]. The development of varieties able to tolerate calcareous soil conditions would maintain yields and promote a sustainable agriculture. The identification of genetic bases underlying calcareous soil tolerance would help breeders in that purpose. In addition to the general atmospheric  $\text{CO}_2$  increase, extreme climatic events such as heat waves, drought, and flooding are predicted. These climatic factors will likely play a major role in  $\text{CaCO}_3$  dissolution-precipitation, nutrient phytoavailability and *in fine* plant tolerance to calcareous soils. Whether they will aggravate or lift the effects of soil  $\text{CaCO}_3$  is an open question and has been poorly investigated. Here, we first provide an overview of plant strategies at the cellular and molecular levels to tolerate  $\text{CaCO}_3$ -rich conditions. We also review the influence of environmental factors, mainly soil moisture, on nutrient phytoavailability and plant performance in calcareous soils, and we discuss how climate change could impact plant strategies to tolerate calcareous soils. Finally, we propose a strategy to improve plant tolerance to  $\text{CaCO}_3$  using metabolic engineering.

**Suberization:** formation of a complex hydrophobic polyester acting as a physical barrier in cell apoplast of root endodermis.

### Looking for genetic bases involved in $\text{CaCO}_3$ tolerance

In wild plants, selection pressure has driven the emergence and conservation of adaptive responses to calcareous soils, giving rise to **calcicole**, **calcifuge**, and soil-indifferent plant species. Calcicole plants (i.e., plants naturally found in calcareous soils) are characterized mainly by their capacity to mobilize soil P and Fe but also their ability to maintain leaf Fe in metabolically active form [14]. Interestingly, contrasted behaviors exist between species within the same genus [15,16] or species [17], attesting divergent adaptation to local edaphic conditions. It is strongly expected that calcicole species have a natural pool of favorable alleles naturally selected for calcareous soil tolerance that could be exploited in breeding programs [18]. The current dynamics of wild plant genome sequencing, assembly, and publication attests to the interest of the scientific community in genetic resources notably from crop wild relatives [19,20]. Surprisingly, the literature related to genomic bases involved in this trait emerged only recently compared with other edaphic stresses such as salinity [21].

*In situ* collection of calcicole and calcifuge individuals for genome sequencing is the first step of a relevant strategy to identify genomic bases and alleles linked to edaphic divergences. Subsequently, their performance can be evaluated in more controlled environments such as experimental gardens

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vacuolar compartment. Enzymatic steps proposed for metabolic engineering to neutralize  $\text{HCO}_3^-$  and favor the formation of Ca-oxalate crystals are shown in red. The C-shape forms in light gray and dark gray represent coumarins and phytosiderophores, respectively. Coumarins are secreted via PDR9, and the complex coumarin- $\text{Fe}^{3+}$  is imported in the cytosol via an unknown mechanism named Cl [61]. Phytosiderophores are exported via the complex coumarin- $\text{Fe}^{3+}$ , which is imported in the cytosol via YS1/YSL transporters. Figure created with [BioRender.com](https://www.biorender.com). Abbreviations: ACA, autoinhibited  $\text{Ca}^{2+}$ -ATPase; C, cytosol; Ca, calcium ion  $\text{Ca}^{2+}$ ; CA, carbonic anhydrase;  $\text{CaCO}_3$ , calcium carbonate; CAX,  $\text{Ca}^{2+}/\text{H}^+$  exchanger; Cl, coumarin importer; CML, CaM-like protein; CNGC, cyclic nucleotide-gated channel;  $\text{CO}_2$ , carbon dioxide; CW, cell wall; GLR, glutamate receptor;  $\text{H}^+$ , proton;  $\text{HCO}_3^-$ , bicarbonate; OA, organic acid; P, phosphate ion  $\text{HPO}_4^{2-}$ ; PDR9, pleiotropic drug resistance 9; ATP-binding cassette-type transporter PDR9/ABCG37; PEPC, phosphoenolpyruvate carboxylase; SULTR1;1, sulfate transporter 1;1; TOM1, transporter of mugineic acid family phytosiderophores 1; TPC, Ca influx channel two-pore channel; V, vacuole; YS1, yellow stripe 1; YSL, yellow stripe 1-like.

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or pots filled with calcareous and noncalcareous soils. Quantitative genetic approaches such as **genome-wide association studies** (GWASs) can be applied to the collected phenotypic data to identify genomic bases involved in plant adaptation. Recently, inter- and intraspecific genomic comparisons of natural populations growing on calcareous and noncalcareous soils revealed candidate genes for  $\text{CaCO}_3$  tolerance in wild Brassicaceae species *Arabidopsis lyrata* [22], *Microthlaspi erraticum* [23], and *Arabidopsis thaliana* [24,25], as well as in Juglandaceae *Platycarya* species [26]. It is noteworthy that *A. thaliana* recently emerged as a model plant to characterize calcareous and saline calcareous soil tolerance (Box 1).

The transcriptomic response to sodium bicarbonate ( $\text{NaHCO}_3$ ) has increasingly been documented in recent years, notably in *A. thaliana* (see below) and in crops such as grapevine [27], cotton [28], and industrial hemp [29]. Several studies compared the effect of high pH (using different buffers) and  $\text{NaHCO}_3$  on *A. thaliana* transcriptome to identify genes specifically mobilized in response to  $\text{HCO}_3^-$  [30–32]. Overall, they revealed that  $\text{NaHCO}_3$  treatment worsens plant performance and induces more differentially expressed genes than high pH, highlighting the complexity of plant response to bicarbonate stress. Gene ontology analysis revealed notably an enrichment in iron, reactive oxygen species, but also energy-related terms [31].

### Impact of $\text{HCO}_3^-$ on plant nutrition

$\text{HCO}_3^-$  is considered the main cause of chlorosis [12]. Indeed, its high buffering effect increases pH in root apoplast and disturbs nutrient absorption. Moreover, it induces xylem sap alkalization and Fe immobilization in root and leaf apoplast [12,33]. In calcareous soils,  $\text{HCO}_3^-$  concentration ranges from 5 to 35 mM [30].  $\text{HCO}_3^-$  is not freely permeable to membranes, so its import in cells requires transporters [34]. Cyanobacteria possess a Na-dependent  $\text{HCO}_3^-$  symporter [35]. Although no specific transporters have been identified in plants,  $\text{HCO}_3^-$  could compete with other anions for transport through anionic channels and transporters [36] and affect transporter activities, as demonstrated in guard cells for the SLOW ANION CHANNEL-ASSOCIATED 1 (SLAC1) anion channel [37]. The  $\text{HCO}_3^-$  effect on root nutrient uptake has been studied in maize, poplar, and

#### Box 1. *Arabidopsis thaliana* ion homeostasis in calcareous soils

*A. thaliana* is considered as a moderately calcifuge species because natural populations can be found in a wide gradient of  $\text{CaCO}_3$  content in soil [17]. The comparison of these populations' performance in siliceous and calcareous experimental gardens helped to characterize  $\text{CaCO}_3$  influence on nutrient acquisition [17]. When grown in calcareous soil, calcicole individuals perform better in terms of biomass production and fitness than calcifuge ones. The comparison of rosette ionome of individuals grown in noncalcareous and calcareous experimental gardens revealed that calcareous soil induced a global decrease in Fe and Mg but an increase in Ca and P. Concerning the other elements, their change varies according to  $\text{CaCO}_3$  content in their origin soil. For example, Na increased in individuals native from low to intermediate calcareous soils but decreased in the ones issued of highly calcareous soils. Interestingly, rosette contents in K, Mn, and Zn were relatively stable in spite of the expected reduced availability of these elements as pH increases [8].

In addition to elevated carbonate content, saline calcareous soils are enriched in sodium, which is the main exchangeable cation. Recently, a GWAS allowed the identification of candidate genes for saline calcareous soil tolerance [24]. Allelic variation in *AMINOPHOSPHOLIPID ATPASE 3* (*ALA3*) gene was linked to leaf Fe, whereas allelic variation in *NOVEL INTERACTOR OF JAZ* (*NINJA*) and *YUCCA8* (*YUC8*) to leaf Na. *ALA3* likely ensures the trafficking of auxin transporter in response to Fe deficiency [78]. *NINJA* regulates jasmonate response, a phytohormone notably involved in the response to salinity [79]. *YUC8* takes part in auxin biosynthesis and is regulated by methyljasmonate [80]. In a second study, the authors combined genomics, transcriptomics, and bulk segregant analysis sequencing (BSA-Seq) to identify new candidate genes [25]. The phenotyping of knocked-out mutants helped in identifying *DIOXYGENASE FOR AUXIN OXIDATION 1* (*DAO1*) as a serious candidate gene for bicarbonate tolerance. Indeed, *dao1* mutant exhibited a better performance than wild type in terms of relative leaf biomass in response to 10 mM  $\text{NaHCO}_3$  in hydroponics and siliques number in calcareous soil. *DAO1* encodes a 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase involved in tissue-specific oxidative inactivation of auxin [81]. *DAO1* expression decrease could stimulate root growth and help in resources foraging in alkaline soils. Altogether, this study pushes the idea that phytohormones, especially auxin and jasmonate, play key roles in plant tolerance to saline calcareous soil.

elder [38,39]. Using labeled C, several studies demonstrated a possible  $\text{HCO}_3^-$  uptake because labeled C was detected in root and shoot parts, but carbon acquisition by the root system is usually minor compared with the shoot part [36]. It was estimated that DIC uptake *via* the roots contributes to 1%–20% of total C assimilation, depending on the studies [40,41]. However, this part could be significantly increased in calcareous soil and disturb nutrient absorption and assimilation. Plant strategies to regulate  $\text{HCO}_3^-$  absorption and accumulation are poorly known. The activity of enzymes that use  $\text{HCO}_3^-$  as substrate (carbonic anhydrases, phosphoenol-pyruvate carboxylase) could represent a mechanism to modulate the intracellular levels of bicarbonate (Box 2).

### Ca homeostasis regulation in calcareous soil

Calcium is essential for plants as a component of membranes, cell wall, and as a messenger [42]. At the root level, Ca mainly uses the apoplastic pathway to reach the xylem, except in the endodermis, where the Casparian strip imposes the use of the symplastic pathway. It is then transported to the shoot via the xylem to supply organs, depending on their transpiration rates [43]. The maintenance of a low cytosolic Ca concentration is necessary to keep its messenger role [42]. In calcareous soils, the regulation of Ca homeostasis is challenging for plants due to its excess in soil. Indeed, plant efforts to acidify the rhizosphere *via* proton secretion induce  $\text{Ca}^{2+}$  release from  $\text{CaCO}_3$  [44]. The secretion of citric acid also dissolves  $\text{CaCO}_3$ , but Ca is subsequently chelated by citrate, leading to the formation of Ca citrate crystals as shown in the rhizosphere of *Lupinus albus* cluster roots [45]. This mechanism efficiently blocks Ca uptake in calcareous soil. A strategy observed in soil-indifferent Proteaceae species consists in blocking Ca at the root level. **Suberization** and **lignification** of the endodermal cells physically limits Ca access to the xylem and the translocation in shoot parts [15]. In parallel, Ca can accumulate in cortical cells in roots. Such a strategy has never been investigated in the moderately calcifuge species *A. thaliana*, although a Ca exclusion strategy has been suggested [17]. Indeed, when grown in calcareous soil, calcicole populations accumulated less Ca in leaves than calcifuge ones. The sequencing and analysis of **genomic variations** in *Arabidopsis lyrata* calcicole and calcifuge populations highlighted a set of candidate genes linked to edaphic adaptation [22]. Interestingly, a glutamate receptor-like (*GLR2.5*) gene resulting in duplication-fusion events was almost only found in calcicole *A. lyrata*. Members of the GLR family are involved in  $\text{Ca}^{2+}$  homeostasis, but the role and the subcellular localization of *GLR2.5* have never been characterized [46]. Combining genomic and transcriptomic

#### Box 2. How do plants deal with bicarbonate excess?

Little is known about plant strategies to attenuate  $\text{HCO}_3^-$  impact on plant homeostasis in calcareous soils. One option would be to block its absorption and access to xylem, another one to enzymatically neutralize  $\text{HCO}_3^-$ . In plants, few enzymes using  $\text{HCO}_3^-$  as substrate have been identified [36]. Carbonic anhydrase (CA) catalyzes the reversible dehydration of  $\text{HCO}_3^-$  to form  $\text{CO}_2$ , whereas PEPC uses it to carboxylate phosphoenolpyruvate (PEP) and form oxaloacetate. Both enzymes are known mainly for their role in C4 photosynthetic pathways, where they play a key role in C metabolism in mesophyll cells. At the opposite, very little is known about their role in C3 plants and nonphotosynthetic organs [82]. In plants, CA diverge in three types:  $\alpha$ -,  $\beta$ - and  $\gamma$ -types [83]. The six  $\beta$ -CA found in *A. thaliana* exhibit different expression patterns and subcellular localizations. *B-CA1* and *-2* genes are the most expressed in leaves, whereas  *$\beta$ -CA4* and *-5* genes are the most expressed in roots [83]. Applying low  $\text{NaHCO}_3$  concentration to compost significantly increased the expression of five of the six  $\beta$ -CA genes (excepted  *$\beta$ -CA3*), with the highest increase observed for  *$\beta$ -CA1*, as well as carbonic anhydrase activity [84]. This study attests the necessity for plants to deal with  $\text{HCO}_3^-$  influx *in planta*. The induction of  *$\beta$ -CA4* and *-5* likely plays a role in neutralizing  $\text{HCO}_3^-$  at the root level to limit its access to the xylem and translocation to the shoot [84]. By homology with their putative role in  $\text{CO}_2$  homeostasis in legume root nodules [85], CA could be involved in  $\text{HCO}_3^-$ - $\text{CO}_2$  equilibrium in root cells (Figure 1). Four PEPC genes are encoded by *A. thaliana* genome [86]. *PPC3* is clearly expressed mainly in roots, contrary to the other three genes. Interestingly, two PEPCs and their kinases were upregulated, and *PEPC3-1* significantly phosphorylated in response to  $\text{NaHCO}_3$  in grapevine roots [27]. This post-translational modification enhances PEPC activity because the enzyme is less sensitive to feedback inhibition [87]. Moreover, PEPC root activity is quickly and specifically induced by bicarbonate but not iron deficiency or high pH in the calcicole species *Parietaria judaica* [88]. In calcareous soil, we can hypothesize that plants exposed to elevated concentrations in  $\text{HCO}_3^-$  have recruited PEPC to convert it into  $\text{CO}_2$  and decrease pH.

approaches, several actors involved in high Ca adaptation have been highlighted in the karst tolerant *Platycarya longipes* [26]. In addition to two *GLRs*, genes encoding CaM-like proteins (CMLs), autoinhibited  $\text{Ca}^{2+}$ -ATPases (ACAs), Ca influx channel cyclic nucleotide-gated channels, and Ca influx channel two-pore channels (TPCs) have been identified (Figure 1). Altogether, this set of genes fine-tunes Ca homeostasis, controlling cytosolic Ca and its fluxes through cytosolic and vacuolar compartments. To maintain a low Ca concentration in the cytosol, the excess of Ca can be stored in two compartments: the apoplast, where Ca notably interacts with pectin and modulates cell wall rigidity, and the vacuole, which is the main Ca store [43] and where it can interact with C-based molecules such as  $\text{HCO}_3^-$ , oxalate, and other organic ligands [47].

### Carbon calcium inclusions beyond Ca homeostasis regulation

**Calcification** is common in shells or corals, and it has been observed in few plant species. In the plant kingdom, the most frequently observed **carbon calcium inclusions** (CCal) are Ca oxalate crystals and, to a lesser extent,  $\text{CaCO}_3$  [48]. The latter has been observed in different leaf cell types, depending on species such as **idioblasts** in mulberry [49] and trichome head cells in tobacco [50]. CCal dissolution-precipitation reactions help to control Ca homeostasis and, in the case of  $\text{CaCO}_3$ , to buffer the pH. Other biological functions have been attributed to CCal, such as metal detoxification [50] and light collection [51]. Further biological roles have been inferred to  $\text{CaCO}_3$  from what has been observed for Ca oxalate [48]. Interestingly, it was demonstrated that Ca oxalate is an internal source of  $\text{CO}_2$  that plants can remobilize in case of limited  $\text{CO}_2$  influx due to stomatal closure [52]. Consequently, the CCal leaf pool is useful in the context of soil water deficit. Indeed, the plant could save water by limiting stomatal opening without affecting its photosynthetic activity because CCal would supply  $\text{CO}_2$  to the RubisCO. This mechanism could be exploited notably to improve crop water use efficiency (i.e., carbon assimilation per unit of transpired water in drought condition). CCal formation is likely favored by Ca-rich environments such as calcareous soils and would deserve further investigations.

Although CCal have been observed mainly in photosynthetic parts, some studies reported their presence in roots. Raphide crystals (Ca oxalate) have been observed in root idioblasts in *Actinidia* species [53] and grapevine [54]. Crystal formation occurs in individual vacuoles that then fuse into the central vacuole. Idioblasts size, structure, and localization change, depending on the root zones [54]. A recent study suggested that the calcification process could also occur in living roots, mainly in cortical cells but also in vascular tissues to a lesser extent, and continues after their death [55]. According to the equation  $\text{Ca}^{2+} + \text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{H}^+$ , the vacuolar calcification not only alleviates Ca toxicity in cells but also favors the release of protons in the cytosol [53]. This pool of cytosolic protons can be reinvested as **root exudates** to acidify the rhizosphere. In other words, *in planta* calcification could contribute to cope with Ca excess and resource foraging (Figure 1). Vacuolar exchangers such as  $\text{Ca}^{2+}/\text{H}^+$  exchanger (CAX) likely play a key role in vacuolar calcification [56].

Although *A. thaliana* does not accumulate substantial amounts of CCal [57], their formation in the vacuole might help in buffering Ca excess. It is noteworthy that its genetic transformation with two genes involved in oxalic acid biosynthesis from the phytopathogen *Burkholderia gluma* successfully led to the production of Ca oxalate, paving the way for genetic engineering in the model plant to improve  $\text{CaCO}_3$  tolerance (Box 3).

### Acquisition, mobilization, and transport of nutrients

In calcareous soils, plants have to deal with not only Ca excess but also the low nutrient availability induced by interaction with Ca and/or alkaline conditions [8]. Chemically, high pH values will negatively affect the availability of micronutrients such as Fe and Mn due to their high reactivity with hydroxide ions ( $\text{OH}^-$ ) and formation of poorly soluble Fe and Mn oxides [58]. Grasses have

### Box 3. Improving CaCO<sub>3</sub> and climate change tolerances

The ideal plant to tolerate CaCO<sub>3</sub> would be able to deal simultaneously with Ca and HCO<sub>3</sub><sup>-</sup> excesses. This could be achieved in a two-step enzymatic process. The first enzyme, PEPC, catalyzes the following reaction: PEP + HCO<sub>3</sub><sup>-</sup> → Pi + oxaloacetate (Figure 1). This first step would allow regulation of pH alkalization by consuming HCO<sub>3</sub><sup>-</sup>. The second step consists in converting oxaloacetate into oxalate with bacterial genes from the oxalate biosynthetic operon [57]. Engineered plants accumulate Ca oxalate crystals in the vacuole of mesophyll cells. In plants, several pathways for oxalate biosynthesis are possible, and ascorbic acid is the most probable precursor, although the gene(s) involved have not been identified [69,89]. The gene encoding an enzyme able to directly convert oxaloacetate into oxalate also remains to be discovered in plants. The machinery involved in the formation of Ca oxalate crystals seems to be conserved [57] and could be potentially used by calcifuge crop species to accumulate them. It would be especially useful when rainfall events increase HCO<sub>3</sub><sup>-</sup> content in the calcareous soil solution. In dry conditions, Ca oxalate will constitute a useful CO<sub>2</sub> pool to supply RubisCO when stomata are closed and external CO<sub>2</sub> entry is limited (e.g., high temperature and/or soil water deficit). This mechanism could also help plants in their adaptation to atmospheric CO<sub>2</sub>, which is predicted to gradually increase.

Preliminary tests could be done in the moderately calcifuge model plant *A. thaliana* before transferring to crops. We do not expect oxalate to be secreted in soil for resource foraging as calcifuge species, contrary to calcicole ones; rather, it can be used to form Ca oxalate and combat Ca excess *in planta* [70]. However, a potential risk would be oxalate degradation by different endogenous enzymes [89]. Moreover, PEP, which is provided *via* the glycolysis pathway, is also a precursor for the biosynthesis of phenolic compounds and aromatic amino acids *via* the shikimate pathway. Hence, it will be necessary to check if forcing C flux toward oxalate does not induce detrimental effects on plant metabolism and performance. Although engineered Ca oxalate accumulating *Arabidopsis* exhibit a reduction in plant stature compared with wild type in optimal growth conditions [90], it would be interesting to compare their performance in CaCO<sub>3</sub>-rich substrate, water deficit, and combined stresses conditions.

developed a strategy based on the secretion of phytosiderophores (PS) and absorption of Fe-PS complexes that is particularly efficient in alkaline conditions because the Fe-PS complexes are remarkably stable at high pH values [59,60] (Figure 1). In nongraminaceous plants, coumarins were identified as key factors for Fe mobilization through their Fe binding and redox capacities (reviewed in [61]). It is noteworthy that, at alkaline pH, the coumarin metabolism is oriented toward fraxetin that appears to be the most efficient coumarin to sustain Fe mobilization as a transport-competent Fe chelator that would be taken up by roots by an unknown machinery [62].

Soil solution Ca<sup>2+</sup> ions are responsible for the formation of Ca-P minerals that reduce the phosphate pool available for plant nutrition [9]. However, CaCO<sub>3</sub>-rich soil induced an increase in rosette P content, between 50% and 100% in *A. thaliana* populations independently of their original soil [17], reflecting the plant capacity to remobilize the pool of unavailable P in soil. Indeed, plants are able to secrete a large array of root exudates to remobilize unavailable nutrients [61]. Rhizosphere acidification *via* protons or organic acids (mainly citrate, malate, and oxalate) secretion locally reduces soil pH and helps in metals (Fe, Mn, Zn) and P solubilization [63]. In Proteaceae, higher production of cluster roots and release of organic acids improve calcareous soil tolerance [64]. However, these specific organs are found in a limited number of plant families. Another plant strategy to mobilize soil P in calcareous soils may be to absorb more Ca to favor the release of P from Ca-P minerals [65,66]. *In planta*, Ca and P colocalization can lead to insoluble precipitates and toxicity symptoms [64] and can prevent these elements from fulfilling their respective biological roles. Controlling their allocation to the different tissues is necessary to avoid that issue. Increasing Ca supply results in a higher P allocation to palisade mesophyll cells, Ca-P deposits, and necrosis symptoms in calcifuge Proteaceae species [64]. To the contrary, the capacity of soil-indifferent Proteaceae species to limit P increase in mesophyll cells is a successful strategy to avoid P-toxicity effects. In *A. thaliana*, Ca is preferentially stored in the vacuole of mesophyll cells and phosphate in the vacuoles of the epidermis and bundle sheath [43]. This tissue-specific storage is likely controlled by tissue- and membrane-specific transporters, especially vacuolar transporters.

Genomic variations among phosphate homeostasis genes are overrepresented in *A. lyrata* calcicole populations, reflecting the importance to adapt to P deficiency in calcareous soil [22].



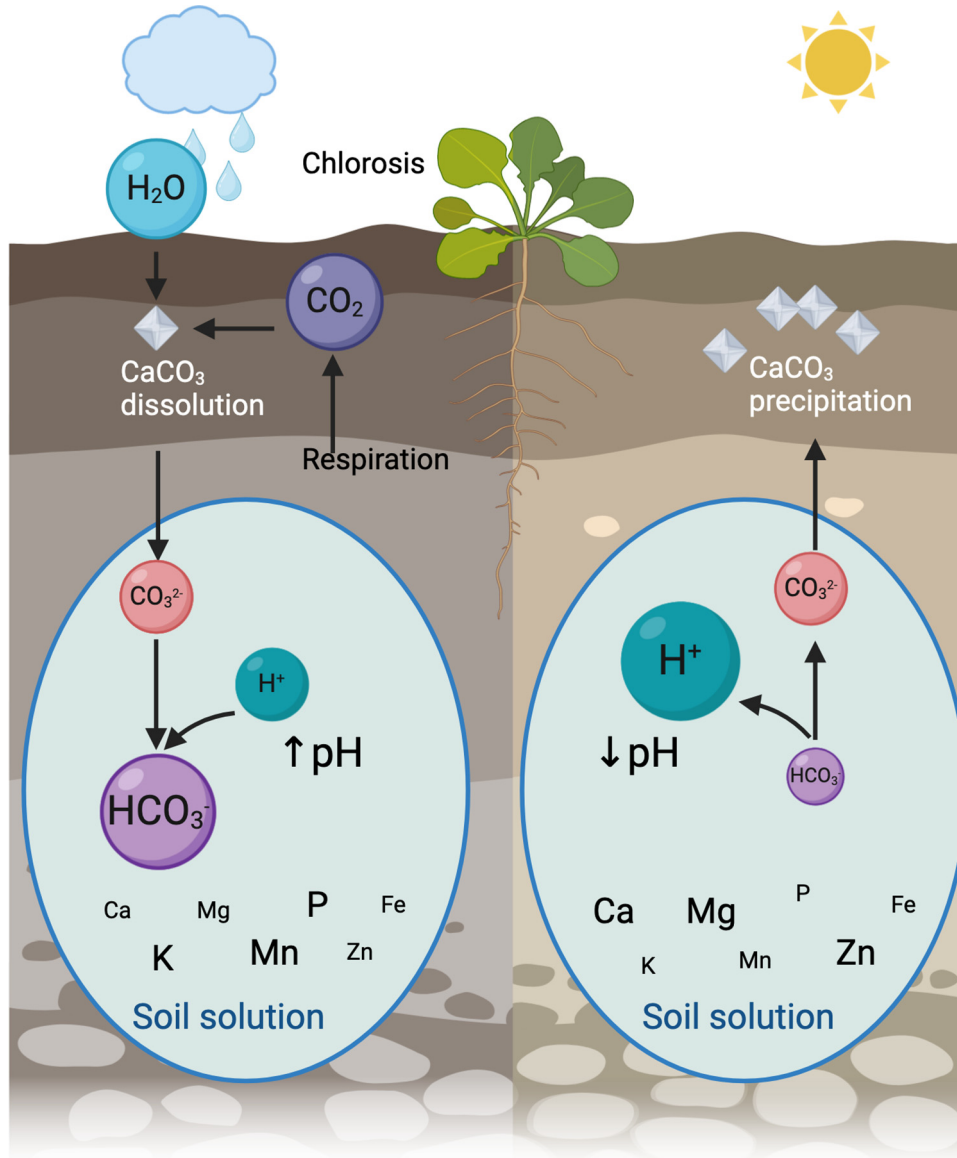
Notably, the gene encoding the vacuolar  $\text{PO}_4$  transporter *VPT1* (or *PHT5;1*) has been identified as a candidate gene for the adaptation to calcareous soil (Figure 1). This transporter, mainly expressed in the roots and young leaves, is essential to tolerate low phosphate conditions [67]. The expression of *VPT1* is strongly induced by P deficiency in roots, and the protein transports  $\text{PO}_4$  in the vacuole, a function that is essential for the regulation of P homeostasis. *VPT1* is also induced by high phosphate in older tissues. Because calcareous soil stimulates phosphate uptake in *A. thaliana* [17], an efficient regulation of P storage in the vacuole might be necessary to limit Ca and phosphate interaction in other cell compartments. *VPT1* likely plays a role in phosphate storage in appropriate tissues, depending on leaf developmental stages to avoid Ca-P interaction in *A. thaliana* mesophyll cells.

Sulfur (S) deficiency is also common in calcareous soils because it can react with Ca to form  $\text{CaSO}_4$ . An intronic deletion in the sulfate transporter *SULTR1;1* has been detected as a characteristic signature of the calcicole *A. lyrata* populations [22]. This gene is expressed in root cells in response to sulfate starvation [68]. Interestingly, *SULTR1;1* exhibits homology with animal anion transporter SLC26, in which some members are able to transport oxalate, and has been proposed as an oxalate transporter because *SULTR* family exhibits a large substrate diversity [69]. This hypothesis is consistent with the fact that calcicole plants secrete more oxalic acid [70]. If the intronic deletion induces a loss of function, oxalate would not be imported in the cell by *SULTR1;1* to favor the rhizosphere acidification.

### Calcareous soil tolerance in the context of climate change

Climate change is expected to impose extreme conditions to plants such as elevated  $\text{CO}_2$  and temperature increase, soil water deficit, or flooding (IPCC 2022). Soil water content (SWC) has been reported to have a major influence on plant symptoms in calcareous soils. However, little is known about the impact of soil and air temperature and  $\text{CO}_2$  concentration on plant performance in  $\text{CaCO}_3$ -rich soils.

The fact that increasing SWC worsens the effects of calcareous soil has been reported for several plant species. In the sensitive soybean variety Corsoy, high SWC decreased dry mass and Fe content but increased chlorosis, P, Mn, Zn, and Cu content [71]. Conversely, the tolerant variety Pioneer produced more biomass in spite of a slight increase in chlorosis, but no significant change in ionome was reported. SWC has an important influence on plant performance in calcareous soils because it modulates nutrient concentration in the soil solution. Its impact is not linear but can be modeled with second-order regression and is nutrient dependent [72]. It appears that soil solution pH,  $\text{HCO}_3^-$ , P, and Mn concentrations are more elevated at high SWC, but Ca, Mg, and Zn are reduced (Figure 2). These trends were similar in shoots for Mn and Zn only, in the two calcicole species *Veronica spicata* (Plantaginaceae) and *Phleum phleoides* (Poaceae). Moreover, SWC increase enriched *V. spicata* ionome (except for Fe), whereas it had little or no effect on *P. phleoides* ionome. SWC increase likely stimulates transpiration and mass flow. This process seems to maintain or even increase the foliar content in elements (here Ca and Mg) in spite of their decrease in the calcareous soil solution. *V. spicata* biomass decreased in the highest SWC conditions, whereas it kept on increasing for *P. phleoides*. Therefore, the capacity of the plant to maintain its shoot ionome homeostasis independently of SWC allows it to benefit from a water supply to produce more biomass. Several factors could explain why plants are more susceptible to  $\text{CaCO}_3$  in high SWC conditions. The reversible reaction  $\text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{Ca}^{2+} + 2\text{HCO}_3^-$  shows that  $\text{CaCO}_3$  dissolution/precipitation is influenced not only by water content but also by  $\text{CO}_2$  concentration [1]. Up to a certain point, SWC increase favors soil microorganism aerobic respiration releasing  $\text{CO}_2$ , which is more retained in high SWC [73]. According to the reaction above, soil solution  $\text{CO}_2$  enrichment favors  $\text{CaCO}_3$  dissolution and the release of  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$ . Ca is mainly



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**Figure 2. Influence of soil water content (SWC) on nutrient phytoavailability in calcareous soil.** In wet condition, soil (plant and microbiota) respiration releases more  $\text{CO}_2$ , which promotes  $\text{CaCO}_3$  dissolution and the release of  $\text{CO}_3^{2-}$  and Ca. Proton interaction with carbonate increases the pool of  $\text{HCO}_3^-$  and soil solution alkalization ( $\uparrow\text{pH}$ ). Elevated SWC increases leaf chlorosis. Dry conditions favor  $\text{CaCO}_3$  precipitation, which consumes Ca and  $\text{CO}_3^{2-}$ .  $\text{HCO}_3^-$  deprotonation favors soil solution acidification ( $\downarrow\text{pH}$ ). Nutrient (Ca, Fe, K, Mg, Mn, P, and Zn) concentration in the soil solution is indicated by symbol size. Figure created with [BioRender.com](https://www.biorender.com).

adsorbed by soil constituents, whereas  $\text{HCO}_3^-$  concentration increases in the soil solution and affects plant performance.

The effect of temperature increase on  $\text{CaCO}_3$  is complex because it involves abiotic and biotic factors. Temperature increase reduces  $\text{CaCO}_3$  and  $\text{CO}_2$  solubility but stimulates soil respiration. Contradictory results have been reported on this topic. It was demonstrated that temperature

increase favors  $\text{CaCO}_3$  recrystallization [74]. On the contrary, other studies in laboratory and field were in line with  $\text{CaCO}_3$  dissolution, as attested by  $\text{CO}_2$  emissions derived from soil inorganic carbon [6]. Calcareous soil temperature increase stimulated both root and shoot vegetative biomass production but also chlorosis in sorghum leaves [75]. Overall, the complexity of temperature effect and the lack of experimental data concerning plant response in calcareous soils make it difficult to predict.

A progressive increase of atmospheric  $\text{CO}_2$  concentration is predicted and will likely affect plant mineral nutrition and homeostasis [76]. Open questions concern the impact of atmospheric  $\text{CO}_2$  increase on plant performance in calcareous compared with  $\text{CaCO}_3$ -free soil, as well as on  $\text{CaCO}_3$  dissolution in soil. *In situ* experiments revealed that  $\text{CO}_2$  enrichment progressively stimulates plant aboveground biomass in calcareous grassland [77]. Because  $\text{CO}_2$  takes part to  $\text{CaCO}_3$  equilibrium, we can hypothesize that the predicted atmospheric  $\text{CO}_2$  increase will likely favor its dissolution and generate even more stressful edaphic conditions for plants.

### Concluding remarks and future perspectives

In addition to other likely causes such as Ca excess and low nutrient phytoavailability in calcareous soils, the hypothesis that  $\text{HCO}_3^-$  excess is the main issue for nutrient uptake and assimilation is confirmed by recent physiological and transcriptomic analyses. Indeed, it induces stronger phenotypic and transcriptomic responses than high pH and mobilizes a specific pool of gene.  $\text{HCO}_3^-$  probably enters into the cytosol *via* transporters with low anion specificity, inducing a competition for absorption with essential anions [36], but that by itself cannot explain its detrimental effect. Its negative effect would rather lie on plant difficulties to counteract its buffering effect in the rhizosphere but also *in planta*. Its metabolism remains largely unknown in plants because no transporter has been identified and the few enzymes involved in  $\text{HCO}_3^-$  assimilation have not been studied in the context of calcareous soil tolerance. Nevertheless, PEPC induction in response to  $\text{NaHCO}_3$  in the roots of several species supports that this enzyme plays a key role to counteract  $\text{HCO}_3^-$  excess. One hypothesis would be that  $\text{HCO}_3^-$  assimilation fuels the tricarboxylic acid cycle and stimulates organic acid secretion in calcicole plants.

The literature provides an interesting list of candidate genes for adaptation to calcareous soils, mainly genes encoding ion transporters. Further wet lab experiments would be necessary to determine if and how these genomic variations affect transporter function and ion homeostasis *in planta*. The study of homologous genes in *A. thaliana*, notably by testing mutants in calcareous soils, could help their functional validation.

Ca oxalate crystals appeared as interesting structures to tolerate both calcareous soils and SWC variations in the context of climate change. Metabolic engineering in *A. thaliana* would allow one to check if these CCal really confer an advantage in calcareous soil at different SWC, temperature, and  $\text{CO}_2$  concentration. Overall, future research should evaluate the impact of environmental factors on plant tolerance to  $\text{CaCO}_3$  to better anticipate on crop breeding strategies aiming to limit yield losses in calcareous soil (see also [Outstanding questions](#)).

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### Declaration of interests

The authors have no interests to declare.

### Outstanding questions

How do plants integrate multiple stresses ( $\text{CaCO}_3$ , high temperatures, drought, elevated  $\text{CO}_2$ , etc.) and elaborate a molecular response?

What is the degree of additivity in the responses to individual stress, and can we identify key hubs that integrate the combination of responses?

What would be the effect(s) of elevated temperature and  $\text{CO}_2$  on the biogeochemical cycle of  $\text{CaCO}_3$ ? Increasing or alleviating the detrimental effects of  $\text{CaCO}_3$  on plants?

What is the role of PEPC to counteract  $\text{HCO}_3^-$  excess in calcareous soils?

Could metabolic engineering, notably the formation of CCal *in planta*, improve plant tolerance to  $\text{CaCO}_3$  and other stresses induced by climate change?

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